

Department of Parasitology and Parasitic Diseases
Veterinary Institute of Puławy
Head: Prof. Dr. Witold Stefański

Wiesław CHOWANIEC

Influence of environment on the development of liver fluke, and the problem of superinvasion and reinvasion in the intermediate host

Wpływ środowiska na rozwój larw motylicy wątrobowej oraz zagadnienie superinwazji i reinwazji w żywicielu pośrednim

In spite of the fact that over 70 years passed from the date of the discovery by Leuckart and Thomas of the intermediate host of the liver fluke, *Galba truncatula*, and although during that period informations were gained on a number of environmental correlations between the parasite and its host, the problem, however, of the influence of the environmental conditions on the dynamics of the development of the parasite in the intermediate host remains still actual and is the subject of interest of many investigators in the whole world.

Leuckart 1882 and Thomas 1883 suggested already that the dynamics of the development of the parasite is above all connected with the thermic conditions. Later studies, conducted by other investigators, confirmed the correctness of observations made by those authors and at the same time proved that there was one more fundamental factor, which influenced the dynamics of the development of larvae of liver fluke in the mud-snail, namely the humidity.

According to Ross 1930, the temperature exerts a considerable influence on the development of larvae, whereby the rise of the temperature accelerates their maturation. Kendall 1949b is of a similar opinion and states that both the speed of the development of larvae of liver fluke and the numerosity of its population (particularly that of rediae and metacercariae) are dependent also on the amount of food assimilated by the snail. Insufficient amount of food available to the snails causes a rapid decrease of the number of the developing parasites and hinders the speed of the development. The same author (1949a) observed that while in the snails not exposed to dryness the whole period of development lasted 3 months, in the snails submitted to dryness the development of the larvae was at that time less advanced and attained only the redia stage. Styczyńska, Jurewicz 1958 studying the influence of dryness on the development of the liver fluke in the intermediate host observed about 10 times lesser number of rediae and a lack, or very low production, of cercariae.

Skvorcov et al. 1936 define generally the time of the development from the egg till the cercaria as 70—100 days and from the miracidium till the cercaria as 50—80 days. Roberts 1950 studying the influence of various temperatures on the time of the development of the parasite in snails reports that at the constant temperature of 25°C it was 38—42 days, at the temperature of 16—19°C 57 days and that the temperature of 5°C is critical, because below it the development is completely checked. According to Kendall 1951 the critical temperature is 10°C. In Davtian's 1956 observations the time required for the development of larvae in the snails kept at 15—17°C is 64—68 days, at 22—23°C 33—34 days, and at 24—26°C 25—27 days. Ollerenshaw 1958, 1959 reports that under the

climatic conditions of England the minimal time required for the development from the egg to the cercaria is 10 weeks (usually 12 weeks) and from the miracidium to the cercaria 8 weeks.

One of the forms of the reproduction of the parasite in the larval stages is its ability to produce the daughter rediae. However, it cannot be stated precisely whether the embryonic cells developing in the rediae give rise to the daughter rediae or to the cercariae. Thomas 1883 supposes that the season of the year is the main factor deciding on the production of the daughter rediae, because their development can be observed only in the warm months. Ross et McKay (according to Roberts 1950) found also only in summer the production of the daughter rediae. The authors explain this fact by the trophic conditions (in summer the snails can nourish themselves intensily) what secures a sufficient amount of food for the increased number of larvae. Some investigators, however, conducting dissections of the mud-snails collected in the field in the various seasons of the year did not observe any production of the daughter rediae (Wibaut 1956), or they found this developmental stage very seldom (Roberts 1950).

It can be concluded from the works of a number of authors that the survival of the winter period by *G. truncatula* depends on many factors, the atmospheric conditions and the substratum of the localities, on which the snail lives, being the fundamental ones. According to Mehl 1932, the snails are capable to preserve life for many months at the temperature of -8°C . Nöller et Schmid (after Mehl 1932) assert that both the mud-snails and the larvae are resistant to low temperatures. Kotlan 1957 reports that under the Hungarian climatic conditions about 5% of *G. truncatula* are capable to survive winter. Sazanov 1957, however, conducting investigations of the possibilities of the overwintering of larvae in the snails, received negative results. (I am not reviewing the data found in older publications, in which the opinion was fairly often expressed that the larvae of the liver fluke were unable to survive winter in the snails).

The informations dealing with the described elements have in our country rather a theoretical character (to rely on data collected in other countries), because problems of this kind were hitherto practically not elaborated in our environmental conditions. In view of this and taking into consideration the fact that the majority of the world investigations have a laboratory character, and that the elements depending on the natural environmental conditions in various geographical latitudes can take different forms, the studies of the influence of the factors of the external environment on the development of larvae of the liver fluke should be conducted in close connection with the native geoclimatic conditions.

The aim of the present work is first of all to describe the influence of the natural climatic conditions, proper to our country, on the dynamics of the development of the larvae of *Fasciola hepatica* in the intermediate host, *Galba truncatula*, in various seasons of the year. In the course of investigations the question arose whether there was a possibility of the development of miracidia of the liver fluke in the snail exposed to a superinvasion and reinvasion. Because this problem is of importance to our informations on the dynamics of the invasion under natural conditions and because in the accessible literature it received hitherto no attention, it was also aimed to solve the problem.

I should like to acknowledge my indebtedness to Prof. Dr. W. Stefański for drawing my attention to this theme and for the constant guidance during this work, and to Docent Dr. E. Żarnowski for his valuable information and advice offered to me.

I. Influence of environment on development of larvae in the intermediate host

Material and methods

The miracidia of the liver fluke were obtained from the culture of eggs collected from gallbladders of sheep strongly invaded with liver flukes. The gallbladders were incised and their content was poured out into large Petri dishes, tap water was added and decanted 6—8 times. When all the eggs sunk, they were collected by a pipette and transferred to small Petri dishes containing a layer of water, of a level not higher than 0.5 cm. The Petri dishes with eggs (every dish contained about 300 eggs) were placed in a thermostat at the constant temperature of 26°C for 14 days. On the 15th day, the Petri dishes were exposed to sun rays. After 20—30 minutes the miracidia started to leave eggs and then they were used for the invasion of snails.

The mud-snails were obtained from pure cultures run in one of the experimental basins (see below). 3,000 specimens of snails, 4—5 mm. long, were collected from natural environments and marked by nitro — varnish. This was made to differentiate the mud-snails collected in the field from those which were hatched from eggs layed in the experimental basins. Such a procedure excluded the possibility of using for the invasion specimens from the natural environment. The snails were placed into the basins and on the next day the laying of cocoons with eggs was observed. After about 4 weeks (depending on the temperature) young mud-snails hatched and after attaining the size of 3—4 mm. they were used for the experiments.

The experimental environment. The observations on the influence of climatic conditions on the dynamics of the development of larvae were conducted in experimental environments. They were established in such a way as to correspond, as far as possible, to the conditions of the natural environment. Such an environment was established on a spacious glade surrounded by trees growing not too densely. Three ditches were dug, one near the other. The dimensions of the ditches were 3.5 m. length $\times$ 1.2 m. width $\times$ 1.0 m. depth. The ditches were surrounded with bricks, covered with a 0.5 cm. layer of cement mortar. The permeability to water was similar to well soaked earth. A layer of soil, 40 cm. thick, from the natural environment was placed on the bottom of every basin. To test whether the type of environment has any influence on the development of the snail, one of basins was filled with a clay substratum and the second one with slime. The third basin was designed exclusively for the culture of the mud-snails and it was provided with a slimy-clay substratum. The surface of the environment in every basin was subdivided into three compartments by means of glass slabs suitably adjusted to the interior walls of the basins, whereby the lower edge of every slab was pressed into the substratum 10 cm deep, and the upper edge reached the height of the basin. In this way every basin was provided with three separate, identical environments. Such an arrangement allowed to locate simultaneously three groups of snails invaded in various periods of time.

To secure the growth of algae which constitute the basic food of snails, the basins were filled with tap water and covered with glass panes. After two weeks the environments were abundantly overgrown with algae. This operation was performed only once (with the exception of the basin in which the mud-snails were cultured, where the humidity was kept for the whole period of the culture), before starting the experiments, while in the course of the experiments the living conditions for the snails depended exclusively on the atmospheric influences.

The invasion of the snails. The snails were selected from a pure culture and marked. 20 snails were placed into each Petri dish, containing large numbers of miracidia. The time of exposure was 10 minutes. After the exposure, the snails were placed into the basin with the clay-slime environment.

The temperature of the environment was measured by the use of a maximal-minimal thermometer. The temperature was measured at the height of 10 cm. above the ground, because when taken at the height of 2 m. above the ground in a meteorological cage it is much lower than that close to the ground.

The data concerning the rainfalls were obtained from the Laboratory of the Agricultural Meteorology and Climatology of the Institute of Soil Cultivation and Fertilization in Puławy, which is located about 2 kilometres from the experimental basins.

The term „young sporocyst” used by me, denotes a sporocyst in which the embryonic cells are small and do not show any differentiation resembling the anlagen of the next generation; „mature sporocyst” denotes a sporocyst with the anlagen of the next generation; „young redia” — denotes redia, in which the embryonic cells are small and do not show any differentiation resembling the anlagen of the next generation; „mature redia” — denotes redia with the anlagen of the next generation; „immature cercaria” — denotes cercaria located in the hepato-pancreas, but still unable to become encysted.

Dynamics of development of larvae in the intermediate host

I period

On the 30th of April 1959, 250 snails were exposed to miracidia of the liver fluke. The examinations of the snails (10 specimens from every type of the environment) were conducted on the next and seventh day, subsequently every seventh day in the following way: the body of the snail was liberated

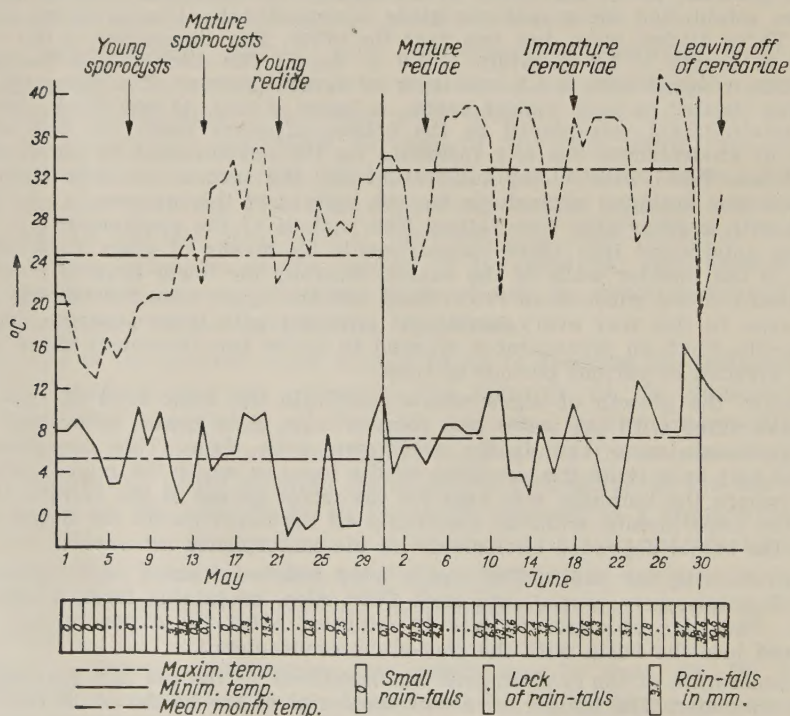


Diagram 1. Time of development of larvae of liver fluke in snails in the first period

from its shell, placed into a small Petri dish containing water and lacerated by preparation needles. The observations were conducted under the dissecting microscope.

The Diagram 1 represents the time of the development of the larvae in two types of the environments with the meteorological data taken into consideration. The full developmental period of the larvae of the liver fluke from the moment of invasion of snails till the liberation of cercariae from them (able to encyst) lasted from the 30th of April till the 2nd of July, it is 63 days.

The number of the individual larvae in the snails was various. The lowest number of the sporocysts in one snail was 2, the highest — 16, the lowest number of the rediae was 9, the highest — 118. Beside the mature sporocysts and rediae, there were also their young forms. The young sporocysts found on the 7th day measured 0.1 mm. in length, the mature sporocysts on the 14th day 0.3 — 0.4 mm., the young rediae on the 21st day 0.6 — 0.7 mm., the mature rediae on the 49th day 1.3 — 1.9 mm. In the majority of the snails with mature cercariae and rediae with cercariae, there were observed also rediae with medium developed germ balls. Calculations of the number of the cercariae developing in the individual snails were not made because in examinations of this kind they would not be accurate.

The life conditions of the snails in the two environments shaped themselves differently. The clay environment, as less permeable to water, was in spite of the scanty amount of rain-falls humid for the major part of the period, even covered with water for some time (June). This resulted in abundant growth of the algae, the snails were not exposed to drought and preserved their full vital activities. The slimy environment, however (with the exception of several days in June), was during the whole period dry, what resulted in poorer growth of the algae and limited the vital activities of the snails.

Although the difference in the formation of the living conditions to the mud-snails in the two environments exerted no influence on the general time of the appearance of the separate larval stages of the liver fluke, it was found that there was an insignificant difference in the number of the developing larvae. In the mud-snails from the clay environment, the average number of the sporocysts in one snail was 8.7 and 54 rediae, while in one snail from the slime environment the average numbers were 6.6 sporocysts and 40.2 rediae.

During this period a total number of 23 mud-snails died in the experimental group (8 snails of the clay environment and 15 of the slime environment).

II period

On the 1st of July 1959, 480 snails were exposed to the miracidia of the liver fluke.

As shown in the Diagram 2, the time of the development of the individual larval stages of the liver fluke is considerably accelerated (in relation to the I period) and the full development of the larvae lasted from the 1st of July till the 4th of August, it is 35 days. The lowest number of the sporocysts found in a snail was 3, the highest 8; the lowest number of the rediae was 11, the highest 64. Similarly to the I period, beside the mature sporocysts and

rediae, there were observed also young forms. The mature sporocysts found on the 7th day were 0.4 — 0.6 mm. in length and on the 14th day the mature rediae measured 1.5 — 2.1 mm. Comparing the general life conditions of the snails in the I and II periods it should be stated that in the II period they were considerably less advantageous. The very high temperature with scanty rainfalls caused a rapid drying of the environments, limiting to a considerable degree the living activities of the snails both in the clay and in the slime environments.

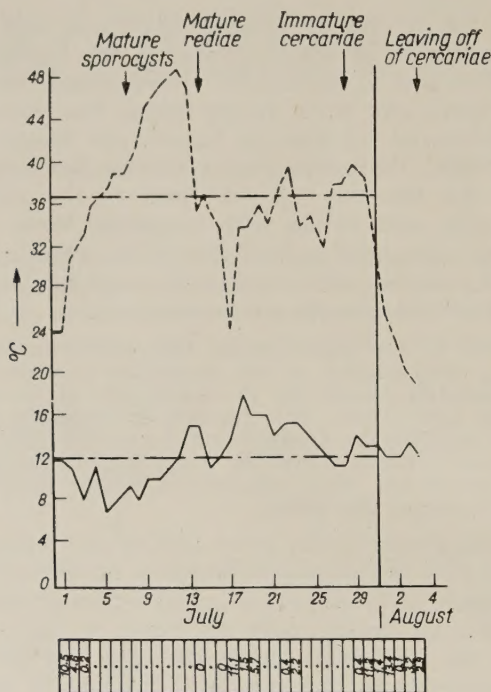


Diagram 2. Time of development of larvae of liver fluke in snails in the second period. Explanations as in diagram 1

In the snails of the clay environment the average number of the sporocysts per a snail was 6.3 and 34.2 rediae, of the slime environment 5.5 sporocysts and 32 rediae.

A total number of 217 snails died in the experimental group (83 in the clay environment and 134 in the slime environment).

III period

On the 21st of July, 540 snails were exposed to the miracidia of the liver fluke.

The early beginning of the investigations in the III period already on the 21st of July, it is at the time when the observations of the II period were

not yet completed, was prompted by the foreseen technical difficulties in the collection of the experimental material (the eggs of the liver fluke).

The Diagram 3 represents the time of the development of the larvae. The full developmental period lasted from the 21st of July till the 8th of September, it is 50 days. The lowest number of the sporocysts was 7, the highest 11, and the lowest number of the rediae was 5, the highest 72. Beside the mature sporocysts and rediae, there were also young forms. The mature sporocysts were on the 7th day 0.3 — 0.4 mm. in length, the young rediae 0.2 — 0.3 mm., the mature rediae on the 22nd day 1.0 — 1.6 mm. Out of the total number of 20 dissected mud-snails on the 29th day from the date of their invasion, in 9 of them were observed beside the rediae with cercariae also rediae with the daughter rediae.

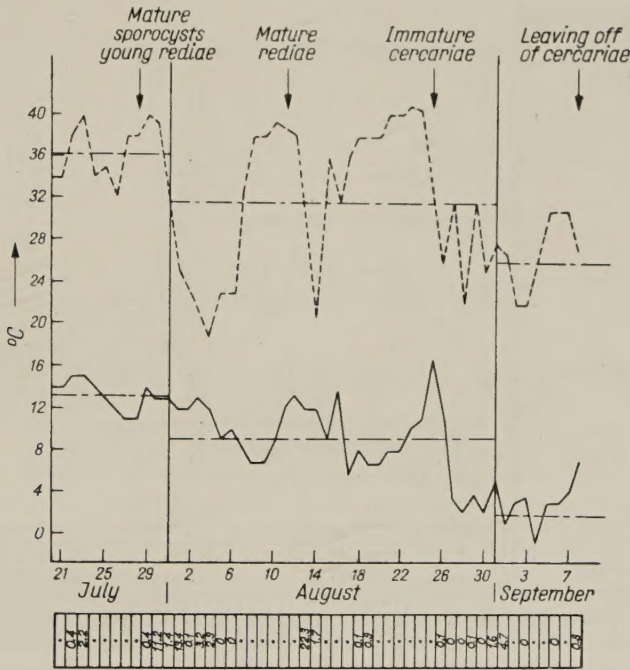


Diagram 3. Time of development of larvae of liver fluke in snails in the third period. Explanations as in diagram 1

The clay environment was humid for 17 days, the slime environment for 9 days. In the snails of the clay environment the average number of the sporocysts in one snail was 9.5 and 52.6 rediae, of the slime environment 8.6 sporocysts and 44.2 rediae.

A total number of 123 snails died in the experimental group (46 of the clay environment and 77 of the slime environment).

IV period

On the 13th of September 1959, 620 snails were exposed to the miracidia of the liver fluke.

As represented in the Diagram 4, the development of the larvae was very slow and beginning from November it was completely checked. In all the snails dissected in that month only young rediae were found, whereby no growth in the length could be observed in them neither any progress in the development of the germ balls. The lowest number of the sporocysts in the snails was 3, the highest 11, the lowest number of the rediae was 9, the highest 59. The young sporocysts found on the 7th day were 0.1 mm. in length, the mature sporocysts on the 21st day were 0.3 — 0.4 mm. in length, the young rediae on the 48th day 0.3 — 0.5 mm. In three out of the 20 examined snails on the 21st day from the moment of the invasion there were observed several sporocysts, which passed double and triple division.

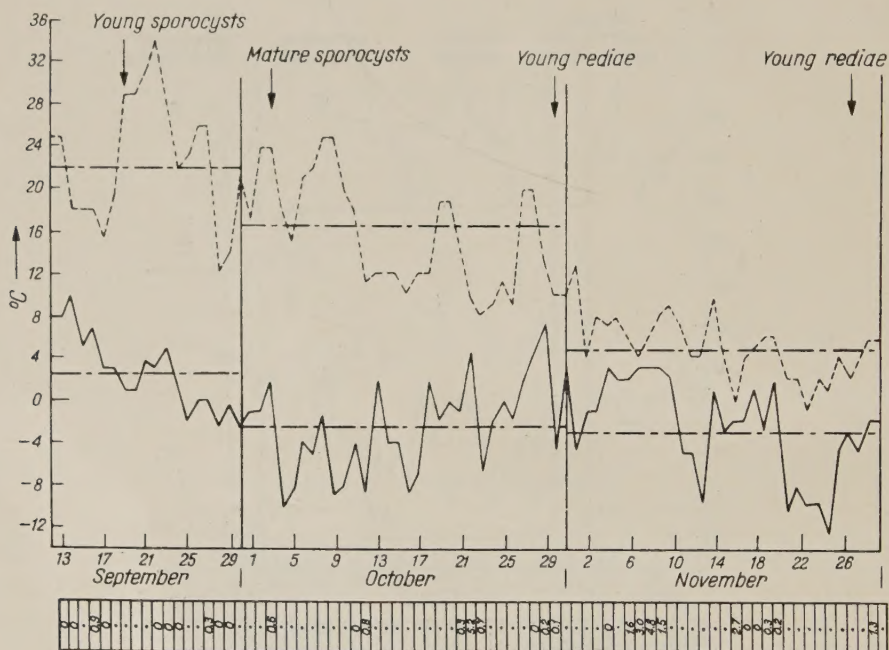


Diagram 4. Time of development of larvae of liver fluke in snails in the fourth period. Explanations as in diagram 1

The formation of the conditions of life for the mud-snails was in this period unfavourable. Very low rainfalls accompanied by a simultaneous drop of the temperature caused an inhibition of the growth of the algae and a decrease of the activity of the snails. The average number of the larvae in one snail in the clay environment was 5.2 sporocysts and 29.1 rediae, in the slime environment 5.4 sporocysts and 28.7 rediae.

A total number of 132 snails died in the experimental group (62 in the clay environment and 70 in the slime environment). From the series of snails invaded on the 13th of September, 198 specimens remained in the experimental environments for the winter period.

V period (continuation of IV period)

The average monthly temperature of winter 1959/1960 was as follows: December: maximal plus 0.6°C, minimal minus 3.5°C; January: maximal minus 1.1°C, minimal minus 7.5°C; February: maximal minus 0.5°C, minimal minus 8°C; March (the first decade): maximal plus 3.5°C, minimal minus 4.7°C. The temperature of the second decade of March and that of the next months are shown in the Diagram 5.

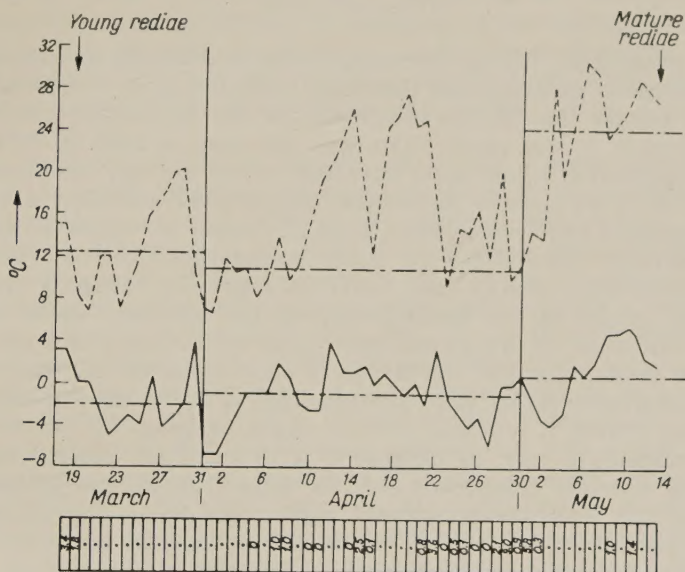


Diagram 5. Time of development of larvae of liver fluke in snails in the fifth period (continuation of the fourth period). Explanations as in diagram 1

The first appearance of the active snails after the winter was observed on the 18th of March, 1960, though the surface in the basins was still covered with a layer of snow. The snails migrated on the walls of the basin through clefts, which were made by the thawing snow adhering to the walls of the basin. Out of the total number of 198 specimens left in the environments for the winter period, 16 snails (8%) survived.

The dissections of the snails which survived the winter were made every 7th day (the first examination on the 18th of March), using each time only one snail. It was aimed to prolong in this way the observations of the progress of the development of the larvae if possible for the longest period of time. Because not all the mud-snails showed the presence of the parasites (lack of invasion in seven specimens), it was impossible to observe the whole life cycle of the larvae and the observations terminated on the 13th of May, 1960, for want of snails.

The young rediae found on the 18th of March measured 0.3—0.4 mm. in length, and on the 13th of May the mature redia measured 1.1—1.4 mm. The number of the rediae (the survival of the winter by only two snails in the

slime environment made it impossible to draw a comparison of the number of the developing parasites in the specimens living in the slime and clay environment) found in the V period fluctuated from 9 to 17 specimens. The fact should be stressed that in every examined mud-snail in this period beside the normal rediae also some rediae (6—9) were found which were already incapable to develop further.

Discussion

Thermic conditions. Taking into consideration only the maximal temperature both the mean monthly and the daily fluctuations, it should be expected in the first period that the development of the larvae of the liver fluke would be quicker in the snails than was the case in reality. However, the minimal temperatures which were observed in this period, the mean monthly values of which were close to the critical temperature and their daily fluctuations were many a time even lower from it, caused at nights a limitation of the developmental possibilities of these larvae, therefore the development could take place only at day-time, what consequently caused a prolongation of the whole period of the development of the parasite. Quite a different situation is observed in the second period, in which there is a great acceleration of the development of the larvae to such a degree that the full period of their development lasted only 35 days, that is by 28 days shorter than in the preceding period. The cause of this rapid progress of the development was the considerable rise, as compared with the first period, both of the maximal and minimal temperatures. The daily fluctuations of the minimal temperatures and their mean monthly value were above the critical temperature, therefore the development of the larvae was not hindered and could take place uninterrupted during the whole 24 hours of the day.

A similar rapid development of the larvae appeared also at the beginning of the third period, even it was more rapid than in the analogous section of the second period. While in the latter period in the first seven days there developed only mature sporocysts, in the third period already the next developmental stage, the young rediae, appeared. However, analysing the temperature conditions of the first seven days of the two periods it should be stated that they were more favourable in the third period, where the maximal temperature fluctuated between 32—38°C, the minimal 15—11°C, while in the second period these fluctuations were with the maximal temperature 24—39°C, the minimal 12—8°C. This is a classical example which illustrates that already insignificant differences of the temperature within several days are able to accelerate considerably or delay the development of the larvae. Finally, owing to the drop of the temperature which took place in the remaining part of the third period and which caused an instant slackening of the rate of the development of the larvae, the full period of the development of the parasite in third period lasted longer than in the second one.

In the fourth period the speed of the development of the larvae was considerably limited. This resulted from the gradual drop both of the maximal and minimal temperatures. Because the minimal temperatures were already at the beginning of the fourth period below the critical temperature, the

larvae could continue their development only due to the maximal temperatures which were at the beginning of this period still rather high. The moment, however, the maximal temperatures reached in the first days of November the level of the critical temperature, the development of the larvae was completely checked. This was proved by the finding in the dissected snails in the subsequent days of November the still young rediae (that is of the same developmental stage which occurred by the end of October) and the lack in the larvae of any progress in the growth in length and in the development of the germ balls. Such a state was maintained till the spring of the next year. However, even in spring the progress in the development of the larvae was very slow, because the minimal temperatures remained still below the critical temperature, and the maximal temperatures were not yet too high; the young rediae were transformed into mature rediae not before a lapse of 57 days.

To summarize, it can be clearly seen what a great part, as regards the speed of the development of the larvae in the snail, is played by the temperature, particularly by the height of the minimal temperature. It is sufficient that the daily fluctuations are close to the critical temperature and the speed of the development of the parasite is seen to be considerably limited even in case when the maximal temperature is very high. It is evident that when the fluctuations of the minimal temperatures are lower than the critical temperature and when there is a simultaneous drop of the maximal temperature, the speed of the development of the larvae becomes still more hindered. When the minimal temperature is considerably higher than the critical one, the development of the larvae runs without any limitations and its rate is then dependent first of all on the height of the maximal temperature.

Humidity and trophic conditions. My observations confirmed also that suitable humidity and good trophic conditions for the snails exert an influence on the dynamics of the development of larvae of the liver fluke. They proved that in the snails not exposed to dryness and provided with a sufficient amount of food there develops a greater number of the larvae than in the snails which remain under inferior conditions. This correlation can be observed both on the example of the type of the environment and in the separate periods. However, I did not observe that in the snails which are of lower vital activities owing to the lack of humidity, the speed of the development of larvae was hindered. This is proved by the equal rate at which larvae were developing in the snails in the clay and slime environments, though the latter one had as a rule inferior humidity conditions. The next example is the second period. In this period the development of the larvae was most rapid, as observed in the annual cycle, in spite of the fact that this period was characterized by the worst humidity conditions and that the snails had a completely limited vital activity.

This proves that under natural conditions even long lack of humidity can cause only a limitation of the numerosness of the populations of the developing parasites but does not influence the speed of the development. A slackening of the speed of the development can occur only in case when the snails remain throughout the whole time, day and night, in a state of anabiosis. However, because the nights are characterized by a fall of the temperature and the appearance of the dew, the snails become at this time more

active and therefore are able to take food. Thus they can, at least partly, cover their food requirements, what secures to the larvae of the liver fluke sufficient food to provide means for the development even during the period of dryness. This fact is of great importance in the epizootiology of the fluke disease, because it points that the dry periods are as dangerous as the wet ones.

Analysing ultimately the influence of the individual factors (temperature, humidity, food) on the dynamics of the development of the larvae of the liver fluke in the intermediate host it should be stated that the temperature plays under natural conditions the basic and decisive part.

II. Observations on production of daughter rediae and division of sporocysts

The question of the occurrence of the daughter rediae in the course of the development of the liver fluke and the factors which decide of the appearance of this phenomenon are since long time in the sphere of interest of many investigators. In the last decade there appeared in the world literature two positions on this theme. Roberts 1950 conducting dissections of the mud-snails collected in the field was able to find only from time to time the daughter rediae during the period from June till October. Wibaut 1956, however, examined the mud-snails collected from natural environments in various seasons of the year and found no daughter rediae at all.

In the course of the present investigations (independently of the observations made during this work, when also the daughter rediae were observed only in 9 snails) conducted several years, I found the daughter rediae only in about 90 out of about 7,600 dissected snails, most commonly in the summer months, although in several cases (4) also at the beginning of November. Thomas 1883 comparing the rediae producing cercariae with the rediae producing daughter rediae (both larvae originated from the same snail) observed that the latter ones were usually smaller, whereby the length of their pharynx and intestine was longer than in the rediae producing cercariae. The next difference was in the number of the offspring: the number of the daughter rediae produced by the mother rediae was lower (the mother rediae can contain from 1—3 daughter rediae and several germ balls at various stages of the growth) than of the cercariae. In the course of the present observations I found exclusively the difference in the number of the developing daughter rediae and cercariae: in the larvae producing the daughter rediae the number fluctuated from 1—5 plus several germ balls, but the number of the cercariae produced by the larvae was 12—17. However, I did not observe any regularity as regards the length of pharynx and intestine in the larvae producing the daughter rediae or cercariae. Frequently in the rediae producing the cercariae the length of pharynx and intestine was longer than in the larvae producing the daughter rediae and vice versa.

Though the finding of the daughter rediae mainly in the summer months could be an evidence that the main factor deciding on their production is the season of the year, the fact that only in relatively few snails living under the same environmental conditions can be observed daughter rediae offers basis to the supposition that there are some hitherto unknown other factors on which the production of these larval forms is dependent.

Both Leuckart 1882 and Thomas 1883 observed that the well grown up sporocysts of the liver fluke were able to reproduce by way of the transverse division. Their body becomes locally narrower, usually already at the early stage of the growth of the sporocysts, and subsequently a division into two parts takes place. The germ balls located in the sporocyst undergo division into daughter individuals (of the sporocyst type).

On the basis of my observations made in the course of the present work it is evident and deserves to be stressed that the sporocysts of the liver fluke are able to undergo not only a double division, but also a triple division. Thus the parasite can produce still greater number of individuals, what constitutes an additional trump in its struggle for the preservation of species.

III. Possibility of development of miracidium in snails exposed to superinvasion and reinvasion

It can be supposed that the mud-snails living under natural environmental conditions can be very often exposed to superinvasion and reinvasion with the miracidia of the liver fluke. In connection with this the question arose, whether the miracidia, which penetrate into the body of the snail showing invasion with later developmental stages (rediae), or after an undergone invasion, can develop, or whether there appears in the host a resistance which makes the development of the parasite impossible? The lack of publications dealing with this problem in the literature accessible to me was the cause of the initiation of the investigations, particularly that beside theoretical meaning it has also a practical aspect.

For the experiments mud-snails cultured in the experimental environment (pure culture) were used. To obtain a series of snails with later larval forms of the liver fluke and a series of snails, which have undergone an invasion, 390 *Galba truncatula* were exposed to miracidia of the liver fluke. The invasion was brought about in small Petri dishes in which numerous miracidia were placed. The time of exposure was 10 minutes. The snails were next transferred to clay bowls without glazing, containing good environmental conditions (abundance of food and moisture). The temperature of the lodge, in which the bowls with the snails were kept, fluctuated within 16—20°C. After 35 days from the moment of the invasion, when in the snails there were found the rediae with the anlagen of the cercariae and simultaneously an absence of earlier sporocysts (on the basis of the dissections of 50 mud-snails), 200 snails were selected as the series designed for the superinvasion. The remaining 120 snails were used for the reinvasion.

Simultaneously with the repeated invasion of the series of snails, which showed later larval stages of the liver fluke, the same number of mud-snails from pure culture (free from invasion) was invaded. They were the control series.

The snails were invaded in Petri dishes by placing in every dish 20 snails (10 from the individual series) and destining to every snail 15 miracidia. The time of exposure was one hour. The snails were then transferred to the clay bowls containing uniform environmental conditions. The bowls were kept at room temperature (17—20°C). The invaded snails were dissected every

day, examining up to the moment of the finding of the first sporocysts 2 specimens from each series, subsequently 20 specimens from each series. The first sporocysts were found in two snails of the control series on the 8th day following the invasion.

Table I
Results of superinvasion of snails

Successive day of snail autopsy	Series A			Series B		
	Superinvasion of snails			Control snails		
	Number of			Number of		
	autopsies	snails with sporocysts	sporocysts in each snail	autopsies	snails with sporocysts	sporocysts in each snail
8	2	-	-	2	2	5,7
9	20	-	-	20	20	4,7,4,5,5,5,7,7,10,4,6,7,4,4,10,7,6,6,5,6
10	20	1	1	20	20	6,9,6,6,6,6,4,7,5,4,4,7,7,9,9,11,4,5,5,6
11	20	-	-	20	20	6,9,8,14,4,6,6,4,6,7,8,4,2,8,8,6,8,7,6
12	20	-	-	20	20	11,9,4,5,9,9,9,5,5,4,7,7,6,6,5,7,8,8,10,16
13	20	7	1,2,2,2,1,3,1	20	19	9,9,8,4,4,4,8,8,9,16,9,5,5,8,8,5,6,8,8
14	20	3	3,1,1	20	20	4,7,7,7,7,11,14,14,10,9,8,8,8,9,9,6,4,4,6,9
15	20	2	1,2	20	20	6,5,5,4,7,11,10,10,9,9,7,7,8,7,8,6,6,4,4,5
16	20	2	2,3	20	20	12,10,10,4,7,4,6,6,6,6,9,8,9,9,7,7,4,4,5,5

Because the observations on the possibility of the development of the miracidia in the snails exposed to superinvasion could be made only till the stage of the mature sporocyst, it was decided to accept as the criterion of the proper comparative observations the period beginning from the moment of the finding of the first sporocysts and terminating with the finding of the first rediae in the control snails.

The first rediae in the control snails were observed on the 17th day following the date of the invasion. The results of this experiment are shown in the Table I.

As shown in the Table, in the control snails exposed to primary invasion, the development of the miracidia took place in almost all individuals (with the exception of one which did not become invaded), while in the snails exposed to superinvasion the development of the miracidia was found only in 15 mud-snails (9.25%). Also the number of the sporocysts, which developed in the snails of the control series, was always higher than in the snails invaded for the second time.

These results entitle to state that the miracidia of the liver fluke, which penetrate into the body of the mud-snail invaded already with later larval stages of this parasite, have minimal chances of further development. It is most probable that the cause which makes impossible the continuation of the development is an immunological phenomenon. The answer to the question with what kind of immunity are we dealing can be given not before suitable studies are conducted.

It was not possible to complete the observations on the possibility of the development of the miracidia in the mud-snails which have undergone an invasion, because all the snails died in the period when the cercariae were leaving their body.

The cercariae started to leave the body of the snails on the 50—52 day following the invasion. The first cases of the death of the snails were observed already on the 6th day from the moment the larvae started to leave the snails, then a new group of dead individuals was found every few days. Postmortem examination of the dead snails revealed in them still a certain number of rediae and cercariae. It seems that the mass dying of the snails in the period of the hatching of cercariae is caused by the latter which by their numerous location in the hepato-pancreas of the snail bring about a serious damage to the organ and consequently a fatal termination.

Although this situation made impossible to get a series of the snails for the planned experiments on the reinvasion, it contributed, however, to the drawing of the conclusion that if not all the snails then certainly the overwhelming majority of them dies at the time when the cercariae start to leave their body or after their emergence. Most probably this indicates that the reinvasion of the snails with the larvae of the liver fluke is in the nature of no practical significance.

Conclusions

1. Out of the three main factors having an influence on the dynamics of the development of the larvae of the liver fluke in the intermediate host, the basic and decisive part is played under natural conditions by the temperature.

2. Under the Polish geoclimatic conditions the time duration of the development of the parasite from the miracidium to cercariae lasts in summer on the average about 55 days, the shortest is about 35 days. From November till the middle of March the development of the larvae is checked.

3. The dryness exerts an influence only on the limitation of the numerosness of the populations of the developing larvae, but it does not decrease the speed of their development.

4. Under the Polish climatic conditions the production of the daughter rediae is a rare phenomenon what gives basis for the assumption that on their production, beside the seasons of the year, also some other factors exert an influence.

5. The sporocysts of the liver fluke can undergo not only a double division but also a triple division.

6. The miracidia, which penetrate into the body of the mud-snail already previously invaded with later larval stages of this parasite (superinvasion), have minimal chances of their further development. Most probably the immunological phenomena render impossible the continuation of the development of the miracidia.

7. If not all the snails then certainly the overwhelming majority of them dies at the time when the cercariae start leaving their body, or after their emergence. In connection with this, most probably the reinvasion of the mud-snails with the larvae of the liver fluke is in the Nature of no practical significance.

Author's address:
Instytut Weterynarii
Puławy

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STRESZCZENIE

Przeprowadzono badania nad wpływem naturalnych warunków klimatycznych, właściwych dla naszego kraju, na dynamikę rozwoju form larwalnych *Fasciola hepatica* w żywicielu pośrednim, ślimaku *Galba truncatula*, w różnych porach roku, oraz nad możliwością rozwoju miracydiów motylicy wątrobowej w błotniarce moczarowej poddanej superinwazji i reinwazji. Ponadto poczyniono obserwacje odnośnie wytwarzania przez redie macierzyste redii potomnych i podziału sporocyst motylicy wątrobowej.

Na podstawie otrzymanych wyników autor dochodzi do następujących wniosków:

1. Z trzech głównych czynników (temperatura, wilgotność, warunki troficzne dla żywiciela pośredniego), posiadających wpływ na dynamikę rozwoju larw motylicy w żywicielu pośrednim, podstawową i decydującą rolę w warunkach naturalnych odgrywa temperatura.

2. W warunkach geoklimatycznych Polski czas rozwoju pasożyta od miracydium do cercarii wynosi w okresie letnim przeciętnie około 55 dni, najkrócej zaś około 35 dni. Od listopada do połowy marca rozwój larw jest wstrzymany.

3. Susza wpływa jedynie na ograniczenie liczebności populacji rozwijających się larw, natomiast nie zmniejsza szybkości ich rozwoju.

4. W polskich warunkach klimatycznych wytwarzanie przez redie macierzyste redii potomnych jest zjawiskiem rzadkim, co pozwala przypuszczać, że na ich produkcję oprócz pory roku muszą wpływać jeszcze inne czynniki.

5. Sporocysty motylicy wątrobowej mogą ulegać nie tylko podziałowi podwójnemu, ale również podziałowi potrójnemu.

6. Miracydia motylicy wątrobowej, które wnika do ciała błotniarki moczarowej wykazującej już inwazję późniejszymi stadiami larwalnymi pasożyta (superinwazja), mają bardzo minimalne szanse dla dalszego swego rozwoju. Najprawdopodobniej przyczyną uniemożliwiającą kontynuację rozwoju miracydiów są zjawiska odpornościowe.

7. O ile nie wszystkie błotniarki moczarowe, to na pewno przeważająca ich większość obumiera w czasie wychodzenia cercarii lub po ich wyjściu. W związku z tym najprawdopodobniej reinwazja błotniarek larwami motylicy wątrobowej nie ma w przyrodzie praktycznego znaczenia.

